

Amendments to the Drawings:

The attached replacement sheet of drawings includes changes to Fig. 9. This sheet, which includes Figs. 9 and 10, replaces the original sheet 6 of 13 of drawings. In Fig. 9, the y-axis is labeled as “Luciferase Activity” in English instead of Japanese.

REMARKS

Upon entry of the foregoing amendments, claims 1-16 and 18-23 will be pending in this application. Claims 1, 2 and 19 are the only independent claims.

Claims 1, 2, 4, 5, 7-10, 12, 15, 16, 18-23 have been amended, without prejudice or disclaimer. Claim 17 is cancelled, without prejudice or disclaimer.

It is respectfully submitted that all claim amendments made herein are fully supported by the claims and specification of the application as originally filed and introduce no new subject matter. For example, the amendment to claim 18 is at least supported by original claim 17, and the amendments to claim 8 and 9 are at least supported by the examples in the specification. Entry of all of the amendments are respectfully requested.

Initially, Applicant is pleased to note that there are no prior art rejections.

Election/Restriction Requirement

The Examiner has made the previous restriction/election of species requirements final. Claims 20-23 are withdrawn from further consideration as being drawn to a nonelected invention. Claims 1-19 are under consideration to the extent they read on the elected species, i.e., SEQ ID NOs: 4 and 5 and their related sequences.

The claims have been amended to cancel the non-elected species, SEQ ID NOs:23 and 24. However, Applicant respectfully requests the Examiner to rejoin SEQ ID NOs:1 and 2 for examination together with SEQ ID NOs: 4 and 5. The Examiner acknowledges that the elected species includes a nucleotide sequence encoding an amino acid sequence exhibiting 95% or more amino acid sequence identity to the amino acid sequence of SEQ ID NO:4. According to the amino acid sequence search results available at the USPTO Public PAIR website, a copy of which is attached to this Amendment as Appendix 1, SEQ ID NO:4 exhibits 96.2% query match with SEQ ID NO:1 (see result 2 in Appendix 1). In addition, according to the nucleic acid sequence search results available at the USPTO Public PAIR website, a copy of which is attached to this Amendment as Appendix 2, SEQ ID NO:2 exhibits 95.9% query match with SEQ ID NO:5 (see result 2 in Appendix 2). Because of the high degree of sequence similarities between the amino acid sequences SEQ ID NOs:1 and 4, and their coding sequences SEQ ID NOs:2 and 5, SEQ ID NOs:1 and 2 should be considered to be within the elected species.

After entry of the claim amendment, claim 1 is directed to an isolated estrogen receptor alpha gene, which encodes the amino acid sequence SEQ ID NO:1 and can have the DNA sequence of SEQ ID NO:2 (see page 60, lines 5-7), and its related sequences; and estrogen receptor alpha 2 gene, which encodes the amino acid sequence SEQ ID NO:4 and can have the DNA sequence of SEQ ID NO:5 (see page 62, lines 11-13), and its related sequences. The estrogen receptor beta gene, which encodes the amino acid sequence SEQ ID NO:23 and can have the DNA sequence of SEQ ID NO:24 (see page 94, last line, to page 95, line 2), and its related sequences, are cancelled, without prejudice or disclaimer.

Accordingly, examination on the merits of claims that read on SEQ ID NOs: 4 and 5, as well as SEQ ID NOs:1 and 2, and their related sequences is respectfully requested.

Objections

The Abstract of the disclosure has been objected to because it allegedly is written in four paragraphs. The abstract is amended to read as a single paragraph in narrative form. Accordingly, withdrawal of the objection to the Abstract is respectfully requested.

Figure 9 is objected to because the X-axis [sic; y-axis] is labeled in Japanese. A replacement sheet of the drawings is submitted. This replacement sheet, which includes Figs. 9 and 10, replaces the original sheet 6 of 13 of drawings. After entry of the replacement sheet, in Fig. 9, the y-axis is labeled as "Luciferase Activity" in English instead of Japanese. Accordingly, withdrawal of the objection to Fig. 9 is respectfully requested.

Claims 4 and 11 have been objected to because of some informalities. Claim 4 has been amended to recite "wherein a promoter is operably linked....." as suggested by the Examiner. Claim 11 has been amended to recite "a mammalian cell" as suggested by the Examiner. Accordingly, withdrawal of the objections to claims 4 and 11 is respectfully requested.

Claims 1, 2 and 19 have been objected to as reciting non-elected species. These claims have been amended to cancel recitations related to SEQ ID NO:23 or 24. As discussed above, SEQ ID NOs:1 and 2, although not explicitly elected, are within the elected species, thus are properly included in the amended claims. Accordingly, withdrawal of the objection to claims 1, 2 and 19 is respectfully requested.

Claim Rejections under 35 USC § 101

Claims 1, 2 and 17-19 are rejected under 35 USC § 101 because the claimed invention is allegedly directed to non-statutory subject matter.

The rejection of claim 17 is moot in view of the cancellation of claim 17 after entry of the claim amendment.

Claims 1, 2, 18 and 19 are amended to recite that the estrogen receptor gene, the DNA or the estrogen receptor is “isolated”.

Accordingly, withdrawal of the rejection to claims 1, 2 and 17-19 under 35 USC § 101 is respectfully requested.

Claim Rejections under 35 USC § 112, Second Paragraph

Claims 5, 6, 8-14 and 16-18 are rejected under 35 USC § 112, second paragraph, as being indefinite.

The Examiner asserts that claims 5 and 6 are vague and indefinite, because it is unclear how a viral particle can contain a virus.

Claim 5 is amended to recite that the vector is a “viral vector”. The term “viral vector” has a definite meaning in the art, i.e., the genetic material of a virus that has been modified to serve as a vector to deliver genetic materials into a cell of interest in recombinant technology. Applicant respectfully submits that after entry of the claim amendments, claims 5 and 6 are definite.

The Examiner asserts that claim 16 is incomplete, because it is unclear what method step is intended by the recitation of “producing estrogen receptor.”

Claim 16 has been amended to recite “thereby producing the estrogen receptor.” After entry of the claim amendment, “producing the estrogen receptor” is no longer a method step, but a desired result from the culturing step. Applicant respectfully submits that the amended claim 16 is complete and definite.

The Examiner asserts that claims 8, 9 and 10 are vague and indefinite because these claims are directed to transformants, but also recite method steps, and that it is unclear whether these claims are directed to products or methods. The Examiner further asserts that, for claims 8-12, it is unclear if Applicant intends isolated cells or cells transformed *in vivo*. Claims 11-14 are rejected because they depend from the rejected claim 8.

Claims 8 and 9 have been amended to recite that the transformant is “produced by introducing ... into a host cell, wherein the host cell is isolated or cultured *in vitro*.” Claim 10 has been amended to recite the transformant of claim 8 “comprising the estrogen receptor gene in a chromosome of the host cell.” Applicant respectfully submits that after entry of the claim

amendments, claims 8-12 are definite, thus claims 11-14 are also definite because they no longer depend from allegedly indefinite claim 8.

The Examiner asserts that claim 17 is vague and indefinite in reciting “partial nucleotide sequence,” because the specification has not provides a clear definition of this term. Claim 18 is also rejected because it depends from the rejected claim 17.

Without acquiescing to the Examiner’s reasoning, claim 17 is cancelled. The cancellation renders the above rejection to claims 17 and 18 moot.

Accordingly, reconsideration and withdrawal of all rejections of claims under 35 USC § 112, second paragraph, are respectfully requested.

Claim Rejections under 35 USC § 112, First Paragraph

Claims 17 and 18 are rejected under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement.

Without acquiescing to the Examiner’s reasoning, claim 17 is cancelled. The cancellation renders the above rejection to claim 17 moot.

Regarding claim 18, the Examiner asserts that the specification does not teach any limiting structure, rather it teaches one exemplary sequence, and that art of record does not make up for the deficiencies in the specification in specifically delineating which nucleotides encode the estrogen (ligand) binding domain (LBD). Applicant respectfully disagrees.

The specification describes the LBD in sufficient detail which enables a person skilled in the art to make and use the LBD. In addition to the description about LBD as pointed out by the Examiner in the Office Action, the specification also provides specific examples on how to make and use a LBD from both an estrogen receptor alpha claimed in the present claims (Example 5, page 74, at line 23, to page 75, line 7), and a LBD of an estrogen receptor beta claimed in the present claims (Example 16, page 106, line 13, to page 108, line 3). Given the high degree of sequence similarities between estrogen receptor alpha (SEQ ID NO:1) and estrogen receptor alpha 2 (SEQ ID NO:4), one skilled in the art would readily know how to make and use the LBD of estrogen receptor alpha 2 based on the teaching of the present application.

In addition, at the time of the invention, LBDs for many estrogen receptors have been identified and studied. Structural and sequence homologies were observed among the LBDs. Ligands for estrogen receptors were readily available. Various *in vivo* or *in vitro* techniques could be used for ligand binding assays to measure the binding of a ligand to a LBD. In fact,

several years before the present application was filed, in the June 1999 version of the SWISS-PROT/TrEMBL database, there were already about 30 estrogen receptor LBD sequences from different species, omitting those with only partially determined sequences (see Mueller-Fahrnow et al., *Current Opinion in Biotechnology*, 1999, 10:550-556, hereinafter "1999 review paper," which accompanies this Amendment for reference). Crystal structures for a number of estrogen receptors had already been determined then. According to the 1999 review paper, the interspecies homology for the LBD is much greater than the similarity between the overall sequences of alpha and beta receptor subtypes (see page 550, 2nd column, 3rd paragraph). At the time of the invention, the state of the art was such that once the sequence of an estrogen receptor is known, one of ordinary skill in the art would readily predict the LBD of the estrogen receptor.

Claim 18 is directed to the LBD of an estrogen receptor gene of claim 1, which includes the estrogen receptor alpha and alpha 2 genes, and their related sequences. In view of the teaching of the present application, particularly in view of the sequences for the estrogen receptor alpha and alpha 2 genes provided in the present application, e.g., SEQ ID NOs:1 and 4 for the amino acid sequences, and SEQ ID NOs: 2 and 5 for the coding sequences, sequence alignment of the present estrogen receptor alpha and alpha 2 genes with other known estrogen receptor genes can be readily performed. The LBD sequences can be readily predicted based on the sequence alignment. Proteins containing the predicted LBD sequences can be readily made by DNA recombinant technology. The LBD functions of such proteins can be readily tested *in vivo* or *in vitro*. Therefore, a partial nucleotide sequence that encodes for a LBD of an estrogen receptor of the present application is enabled.

Accordingly, reconsideration and withdrawal of the rejections of claims 17 and 18 under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement, are respectfully requested.

CONCLUSION

Reconsideration and withdrawal of all of the objections and rejections are respectfully solicited.

Applicant believes that the present application is now in condition for allowance. Upon finding composition claims allowable, Applicant respectfully requests the Examiner to rejoin

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method claims that include all of the limitations of the allowable composition claims. A Notice of Allowance with respect to all elected composition claims and the rejoined method claims is respectfully solicited.

The Examiner is invited to contact the undersigned attorney for any reason to advance the prosecution of this application.

Respectfully submitted,

KOICHI SAITO

October 26, 2007
(Date)

By:



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ASN/WH/hg
Enclosures

United States Patent & Trademark Office

1/1 ページ

Appendix 1

10/501,227		Estrogen receptor genes and utilization thereof					
Salvage New Search	Application Usage	Publication Priority	Metadata Wrapset	Community Data	Foreign Priority	Published Documents	Address & Attorney/Agent

Supplemental Content - Search Results

This page gives you information about the number of versions associated with the application you request. Use this page to obtain specific version information.

[Prev](#)

Version Number	Item ID	Item Size	See Item ID
1	09323b67800d7a2e147.879		○
1	09323b67800d7a31147.879		○
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10/501,227

Estrogen receptor genes and utilization thereof



GenCore version 6.2

Copyright (c) 1993 - 2007 Bioceleration Ltd.

OM protein - protein search, using sw model

Run on: March 10, 2007, 02:20:32 ; Search time 50 Seconds
(without alignments)
1887.708 Million cell updates/sec

Title: US-10-501-227-1
Perfect score: 2672
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Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 800936 seqs, 185091930 residues

Total number of hits satisfying chosen parameters: 800936

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA_New:*
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8: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US60_NEW_PUB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Match	Query Length	DB ID	Description
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2	2571	96.2	582	6	US-10-501-227-4
3	2469	92.4	627	7	US-11-355-544-31
4	2469	92.4	627	7	US-11-368-891-21
5	2269.5	84.9	581	7	US-11-355-544-23
6	2269.5	84.9	581	7	US-11-368-891-13
7	2206.5	82.6	579	7	US-11-355-544-36
8	2206.5	82.6	579	7	US-11-368-891-26
9	2187.5	81.9	583	7	US-11-355-544-17
10	2187.5	81.9	583	7	US-11-368-891-7
11	2117.5	79.2	574	7	US-11-355-544-28
12	2117.5	79.2	574	7	US-11-368-891-18
13	2100.5	78.6	578	7	US-11-355-544-35
14	2100.5	78.6	578	7	US-11-368-891-25
15	2077.5	77.8	582	7	US-11-355-544-19
16	2077.5	77.8	582	7	US-11-368-891-9
17	2072	77.5	585	7	US-11-355-544-38
18	2072	77.5	585	7	US-11-368-891-28
19	1985	74.3	573	7	US-11-355-544-27
20	1985	74.3	573	7	US-11-368-891-17
21	1949.5	73.0	458	7	US-11-355-544-29
22	1949.5	73.0	458	7	US-11-368-891-19

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25	1617	60.5	569	7	US-11-355-544-25	Sequence 25, Appl
26	1617	2100.5	78.6	578	7 US-11-355-544-35	Sequence 35, Appl
14	2100.5	78.6	578	7	US-11-368-891-25	Sequence 25, Appl
15	2077.5	77.8	582	60.5	569 7 US-11-368-891-15	Sequence 15, Appl
27	1542.5	57.7	581	7	US-11-355-544-30	Sequence 30, Appl
28	1542.5	57.7	581	7	US-11-368-891-20	Sequence 20, Appl
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32	1321.5	49.5	643	7	US-11-368-891-23	Sequence 23, Appl
33	1310	49.0	587	7	US-11-355-544-18	Sequence 18, Appl
34	1310	49.0	587	7	US-11-368-891-8	Sequence 8, Appl
35	1307.5	48.9	587	7	US-11-355-544-21	Sequence 21, Appl
36	1307.5	48.9	587	7	US-11-368-891-11	Sequence 11, Appl
37	1304	48.8	587	7	US-11-355-544-37	Sequence 37, Appl
38	1304	48.8	587	7	US-11-368-891-27	Sequence 27, Appl
39	1303	48.8	594	7	US-11-355-544-26	Sequence 26, Appl
40	1303	48.8	594	7	US-11-368-891-16	Sequence 16, Appl
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42	1302.5	48.7	589	7	US-11-368-891-14	Sequence 14, Appl
43	1299.5	48.6	599	7	US-11-355-544-32	Sequence 32, Appl
44	1299.5	48.6	599	7	US-11-368-891-22	Sequence 22, Appl
45	1296	48.5	595	6	US-10-827-121-12	Sequence 12, Appl

ALIGNMENTS

RESULT 1

US-10-501-227-1

; Sequence 1, Application US/10501227

; Publication No. US20060141560A1

; GENERAL INFORMATION:

; APPLICANT: SAITO, Koichi

; TITLE OF INVENTION: Estrogen receptor genes and utilization thereof

; FILE REFERENCE: 600630-21US (561334)

; CURRENT APPLICATION NUMBER: US/10/501,227

; CURRENT FILING DATE: 2004-07-12

; PRIOR APPLICATION NUMBER: JP 2002-004395

; PRIOR FILING DATE: 2002-01-11

; NUMBER OF SEQ ID NOS: 48

; SEQ ID NO 1

; LENGTH: 506

; TYPE: PRT

; ORGANISM: Blue Gill

US-10-501-227-1

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Matches 506; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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QY      121 RLRKCYEVGMMKGGVRKDRGRVLRDRKRRAGTNDREKASKDLEYKTVPPQDRRKHSSSSS 180
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; CURRENT FILING DATE: 2004-07-12
; PRIOR APPLICATION NUMBER: JP 2002-004395
; PRIOR FILING DATE: 2002-01-11
; NUMBER OF SEQ ID NOS: 48
; SEQ ID| ||||||||||||||||||||||||
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RESULT 2

US-10-501-227-4

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; Publication No. US20060141560A1

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; CURRENT APPLICATION NUMBER: US/10/501,227

; CURRENT FILING DATE: 2004-07-12

; PRIOR APPLICATION NUMBER: JP 2002-004395

; PRIOR FILING DATE: 2002-01-11

; NUMBER OF SEQ ID NOS: 48

; SEQ ID NO 4

; LENGTH: 582

; TYPE: PRT

; ORGANISM: Blue Gill

US-10-501-227-4

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; ORGANISM: Blue Gill

US-10-501-227-4

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RESULT 3

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US-11-355-544-31
; Sequence 31, Application US/11355544
; Publication No. US20060183159A1
; GENERAL INFORMATION:
; APPLICANT: Zhao, Huimin
; APPLICANT: Chen, Zhilei
; TITLE OF INVENTION: METHOD FOR ENGINEERING A PROTEIN BY IN VITRO COEVOLUTION
; FILE REFERENCE: IL0013US
; CURREPLHNSMAVQNMLDTITDALIHHSQSGCSA 380
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Db      NT APPLICATION NUMBER: US/11/355,544
; CURRENT FILING DATE: 2006-02-16
; PRIOR APPLICATION NUMBER: US 60/654,269
; PRIOR FILING DATE: 2005-02-17
; NUMBER OF SEQ ID NOS: 46
; SOFTWARE: PatentIn version 3.3
; SEQ ID NO 31
; LENGTH: 627
; TYPE: PRT
; ORGANISM: Micropterus salmoides
US-11-355-544-31

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Query Match 92.4%; Score 2469; DB 7; Length 627;
Best Local Similarity 96.9%; Pred. No. 5.7e-198;
Matches 471; Conservative 4; Mismatches 9; Indels 2; Gaps 2;

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United States Patent & Trademark Office

1/1 ページ

Appendix 2

10/501,227

Estrogen receptor genes and utilization thereof

Search New Cases	Application History	Publication History	Image/File Wrapper	Continuity Data	Foreign Priority	Published Documents	Address/ Money/Agent	Supplemental Content
---------------------	------------------------	------------------------	-----------------------	--------------------	---------------------	------------------------	-------------------------	-------------------------

Supplemental Content - Search Results

This page gives you information about the number of versions associated with the application you request. Use this page to obtain specific version information.

Prev

Version Number	Item ID	Item Size	Start Download
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10/501,227

Estrogen receptor genes and utilization thereof

GenCore version 6.2

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(without alignments)
8479.387 Million cell updates/sec

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Scoring table: IDENTITY_NUC
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Post-processing: Minimum Match 0%
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Listing first 45 summaries

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⇒ ②	1674	95.9	3499	11	US-10-501-227-2 Sequence 2, Appli
3	988.6	56.6	996	11	US-10-501-227-3 Sequence 3, Appli
4	482.6	27.6	1788	16	US-11-355-544-1 Sequence 1, Appli

5	482.6	27.6	1788	16	US-11-368-891-1	Sequence 1, Appli
6	481	27.5	1788	16	US-11-355-544-15	Sequence 15, Appl
7	481	27.5	6450	8	US-10-940-774-18	Sequence 18, Appl
8	481	27.5	6450	11	US-10-515-675A-47	Sequence 47, Appl
9	481	27.5	6450	11	US-10-517-155A-25	Sequence 25, Appl
10	481	27.5	6450	14	US-11-289-102-120	Sequence 120, App
11	481	27.5	6450	14	US-11-283-329-91	Sequence 91, Appl
12	481	27.5	6450	15	US-11-346-759-100	Sequence 100, App
13	479.4	27.5	1788	16	US-11-355-544-7	Sequence 7, Appli
14	477.8	27.4	1788	16	US-11-355-544-9	Sequence 9, Appli
15	476.2	27.3	1788	16	US-11-355-544-11	Sequen 2, Appli
3	988.6	56.6	996	11	US-10-501-227-3	Sequence 3, Appli
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5	482.6	27.6	1788	16	US-11-368-891-1	Sequence 1, Appli
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7	481	27.5	6450	8	US-10-940-774-18	Sequence 18, Appl
8	481	27.5	6450	11	US-10-515-675A-47	Sequence 47, Appl
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ALIGNMENTS

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US-10-501-227-5

; Sequence 5, Application US/10501227

; Publication No. US20060141560A1

; GENERAL INFORMATION:

; APPLICANT: SAITO, Koichi

; TITLE OF INVENTION: Estrogen receptor genes and utilization thereof

; FILE REFERENCE: 600630-21US (561334)

; CURRENT APPLICATION NUMBER: US/10/501.227

; CURRENT FILING DATE: 2004-07-12

; PRIOR APPLICATION NUMBER: JP 2002-004395

; PRIOR FILING DATE: 2002-01-11

; NUMBER OF SEQ ID NOS: 48

; SEQ ID NO 5

; LENGTH: 1824

; TYPE: DNA 213298,

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; ORGANISM: Blue Gill
; FEATURE:
; NAME/KEY: CDS
; LOCATION: (74)...(1822)
US-10-501-227-5

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Matches 1746; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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; Publication No. US20060141560A1
; GENERAL INFORM | | | | |
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; APPLICANT: SAITO, Koichi
; TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
; FILE REFERENCE: 600630-21US (561334)
; CURRENT APPLICATION NUMBER: US/10/501,227
; CURRENT FILING DATE: 2004-07-12
; PRIOR APPLICATION NUMBER: JP 2002-004395
; PRIOR FILING DATE: 2002-01-11
; NUMBER OF SEQ ID NOS: 48
⇒ ; SEQ ID NO 2
; LENGTH: 3499
; TYPE: DNA
; ORGANISM: Blue Gill
; FEATURE:
; NAME/KEY: CDS
; LOCATION: (424)...(1944)
US-10-501-227-2

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Qy 181 AGCGGGCTACAGTCCTCTTGTGTTTGTGCGCTCCAGCCCCAGACTCAGCCCCCTTTATG 240
| | | | |
Db 314 AGCGGGCTACAGTCCTCTTGTGTTTGTGCGCTCCAGCCCCAGACTCAGCCCCCTTTATG 373
| | | | |
Qy 241 CACCCGCCCAGCCACCCTATCTGGAACCACTCAACACCCGTCTAC----- 288
| | | | |
Db 374 CACCCGCCCAGCCACCCTATCTGGAACCACTCAACACCCGTCTACAGATGAGTCTGA 433
| | | | |
Qy 289 -----AGGTCTAGTG 298
| | | | |
Db 434 AAGACTGGTTATTAGGAAAAGAAAGGACGGTGGTGACCATGGAGGAGCTGAGGTCTAGTG 493
| | | | |

QY 299 TCCCATCCAGGACAGCCAGTTCCACAGAGAGGACCCAGTGTGCCACCAAGTATGAGTCTCT 358
Db 494 TCCCATCCAGGACAGCCAGTTCCACAGAGAGGACCCAGTGTGCCACCAAGTATGAGTCTCT 553

QY 359 ATAGTGTGGGGAGTCAAGGGCTGGAGCCAGGGGTTTGAGATGGCCAAGGAGATGCGTT 418
Db 554 ATAGTGTGGGGAGTCAAGGGCTGGAGCCAGGGGTTTGAGATGGCCAAGGAGATGCGTT 613

QY 419 TCTGTGCTGTGTGCAGTGAATATGCTCTGGGTACCACTACGGGGTGTGGTCTGTGAAG 478
Db 614 TCTGTGCTGTGTGCAGTGAATATGCTCTGGGTACCACTACGGGGTGTGGTCTGTGAAG 673

QY 479 GCTGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCAGCAA 538
Db 434 AAGACTGGTTATTAGGAAAAGAAAGGACGGTGGTGA 733
Db 674 GCTGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCAGCAA 733

QY 539 CCAATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGA 598
Db 734 CCAATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGA 793

QY 599 AGTGTATGAAGTGGGCATGATGAAAGGAGGTGTTTCGCAAGGACCGTGGCCGTGTTTTCG 658
Db 794 AGTGTATGAAGTGGGCATGATGAAAGGAGGTGTTTCGCAAGGACCGTGGCCGTGTTTTCG 853

QY 659 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 718
Db 854 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 913

QY 719 ACAAACAGTGTCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGTGTCCGGTGGTG 778
Db 914 ACAAACAGTGTCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGTGTCCGGTGGTG 973

QY 779 GAGGAGGAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGG 838
Db 974 GAGGAGGAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGG 1033

QY 839 GTGCCGAGCCCCCAATGCTGTGCTCCCGTCAGAAGCTGAGCCGACCGTACACCGAGGTCA 898
Db 1034 GTGCCGAGCCCCCAATGCTGTGCTCCC 953
Db 794 AGTGTATGAAGTGGGCATGATGAAAGGAGGTGTTTCGCAAGGACCGTGGCCGTGTTTTCG 853

QY 659 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 718
Db 854 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 913

QY 719 ACAAACAGTGTCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGTGTCCGGTGGTG 778
Db 914 ACAAACAGTGTCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGTGTCCGGTGGTG 973

QY 899 CCATAATGACACTACTACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGG 958
Db 1094 CCATAATGACACTACTACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGG 1153

QY 959 CCAAGAAGCTTCCAGGTTTCTGACAGTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1018
Db 1154 CCAAGAAGCTTCCAGGTTTCTGACAGTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1213

QY 1019 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCGGCA 1078
Db 1214 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCGGCA 1273

QY 1079 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1138
Db 1274 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1333

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Qy 1199 AACCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCCTTCTCTT 1258
| | | | |
Db 1394 AACCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCCTTCTCTT 1453
| | | | |
Qy 1154 CCAAGAAGCTTCCAGGTTTCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1213
| | | | |
Qy 1019 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1078
| | | | |
Db 1214 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1273
| | | | |
Qy 1079 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1138
| | | | |
Db 1274 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1333
| | | | |
Qy 1139 TTGTTGAGATCTTCGACATGCTGCTGG 1259 TCTGCACCGGCACAATGGAGCCCCCTCCACAACAGCATGGC
| | | | |
Db 1454 TCTGCACCGGCACAATGGAGCCCCCTCCACAACAGCATGGCAGTGCAGAACATGCTGGACA 1513
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Qy 1319 CCATCACAGACGCTCTCATACATCATATCAGCCAATCAGGATGCTCGGCTCAGCAGCAGT 1378
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| | | | |
Db 1814 GCGGCGTCTCCTCTTCAGTCGGTTCAGTTCAGGACCCCGAGTCAACCACGAGAGCCCCGA 1873
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Qy 1679 GCAGAGGACCCACAGGTCCAGGAGTCTGCACTACGGAGGGTCCCGCTCTGACTGCACCC 1738
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Db 1874 GCAGAGGACCCACAGGTCCAGGAGTCTGCACTACGGAGGGTCCCGCTCTGACTGCACCC 1933
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Ligand-binding domain of estrogen receptors

Anke Mueller-Fahrnow* and Ursula Egner†

Estrogen receptors are multi-domain proteins that interact with other proteins and DNA to fulfil their function: the regulation of transcription. During the past 2–3 years, our understanding of this complex process has increased tremendously as crystal structures of isolated ligand-binding domains in complex with various ligands, as well as co-activator peptides, are now available. The structural information, combined with new data on novel co-activators/co-repressors, muteins and their actions, and novel ligands, allows for the first time the development of detailed theories for the first steps of transcription initiation.

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Abbreviations

DES	diethylstilbestrol
E2	estradiol
ER	estrogen receptor
h	human
LBD	ligand-binding domain
LBP	ligand-binding pocket
NR	nuclear receptor
RXR	retinoic X receptor

Introduction

The estrogen receptor (ER) belongs to the superfamily of nuclear receptors (NRs). It regulates the expression of specific genes and thus plays a crucial role in many processes, such as the control of reproduction and the development of secondary sexual characteristics [1,2]. It has therefore been a target for research within pharmaceutical companies for many years. As a result, thousands of steroidal and non-steroidal ligands to the ER have been synthesized, which act either as agonists, partial agonists or pure antagonists.

NRs are built up by five to six domains serving different functions. Out of these, the ligand-binding domain (LBD; E-domain) is of special interest not only due to its involvement in ligand binding but also in receptor dimerization and in the transactivation process: the carboxy-terminal region of the LBD comprises the transactivation function AF-2 [3*]. Until recently, the structural basis for these functions has only been poorly understood, but during the past few years crystal structures for the human (h)ER α LBD [4,5,6**] and hER β LBD [7], as well as for a number of other NR LBDs, have been determined [8–15]. With these structures at hand, the differences and similarities between hER α LBDs (Egner U *et al.*, unpublished data) and the recently identified hER β [7,16] can be analyzed.

Although the availability of the structures has contributed to our understanding of ER actions, a number of open questions remain, especially concerning the conformational changes that occur upon ligand binding (described below). In this review, we give an overview of new data on estrogen receptor LBDs with a special emphasis on structural aspects. We try to outline the implications of the structure on our understanding of the protein function and point out areas for future research.

Homology with other nuclear receptors

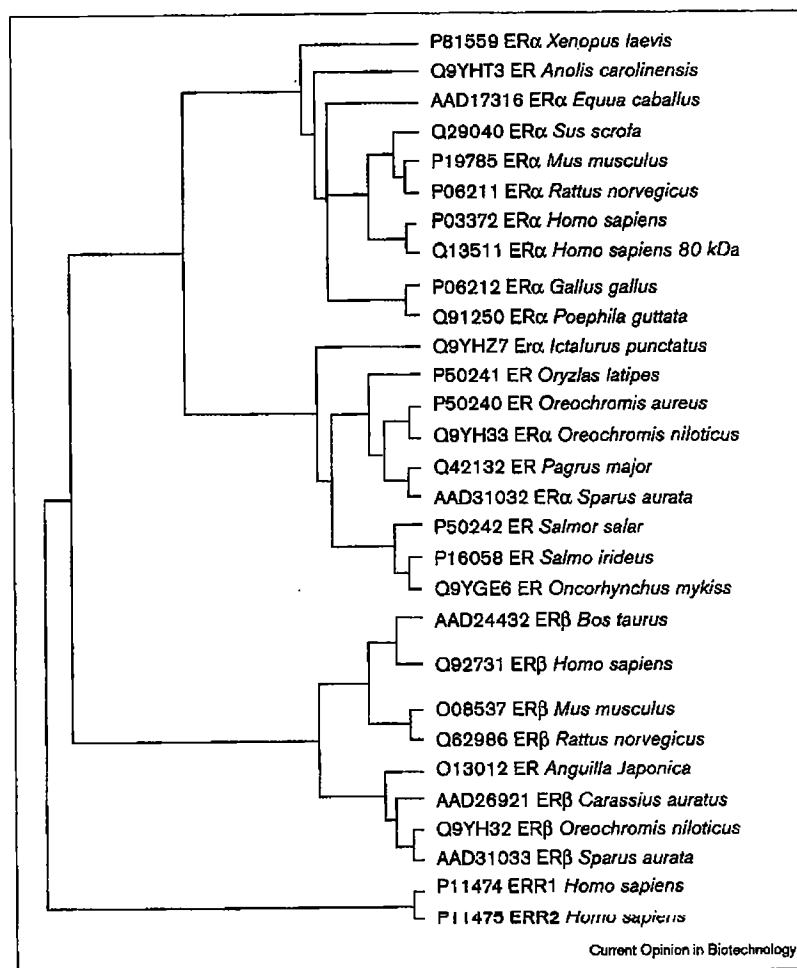
With respect to sequence homology, the superfamily of NRs can be divided into several subfamilies. The ER subfamily comprises ER α and ER β , which share ~55% sequence identity in their LBDs (two proteins are expected to share a similar fold if the sequence identity is >30%, and if the identity is as high as 50% the three-dimensional structures can be assumed to be very similar), and the estrogen-receptor-related orphan receptors ERR1 and ERR2 [17]. On the mRNA level, different splice variants have been identified for ERs, but the corresponding proteins have not been detected [18–23].

In the June 1999 version of the SWISS-PROT/TrEMBL database [24], around 30 ER LBD sequences from different species were found, omitting those with only partially determined sequences. Based on a multiple sequence alignment, a phylogenetic tree was reconstructed (Figure 1). From this presentation, it is obvious that the interspecies homology for the LBDs is much higher than the similarity between the α and β receptor subtypes. It should also be mentioned that for both ER homologues, LBDs from fish and vertebrates can be clearly distinguished with respect to their sequence homologies (Figure 1).

The distribution of conserved residues on the surface of the hER α LBD is shown in Figure 2a. Highly conserved residues within the ER subfamily are indicated by yellow colouring of the protein, which is shown in the same orientation as in Figure 3. A striking feature of the ER subfamily is that most of the conserved residues within the LBD are located in one area of the protein, close to one assumed co-activator binding site (see below). A similar analysis for the progesterone receptor subfamily is given in Figure 2b. The human proteins belonging to this subgroup (progesterone, glucocorticoid, androgen and mineralocorticoid receptors) share ~50% sequence identity within their LBDs, but conserved residues are more equally distributed (Figure 2b). The relevance of this observation is not clear. We know, however, that LBDs function via conformational changes that occur upon ligand binding and by interactions with other ER domains and with co-activators/co-repressors. It can therefore be speculated that a highly conserved region

Figure 1

Phylogenetic reconstruction of the ER LBD sequences (amino acid range as shown in Figure 4) according to the neighbor-joining method as implemented in CLUSTALW V1.7 [49]. Accession numbers referring to the Swiss-PROT/TrEMBL database [24] are given. The ERR1 and ERR2 sequences are defined as outgroup.



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on the protein surface is indicative of a conserved site for protein-protein interactions.

The three-dimensional structure

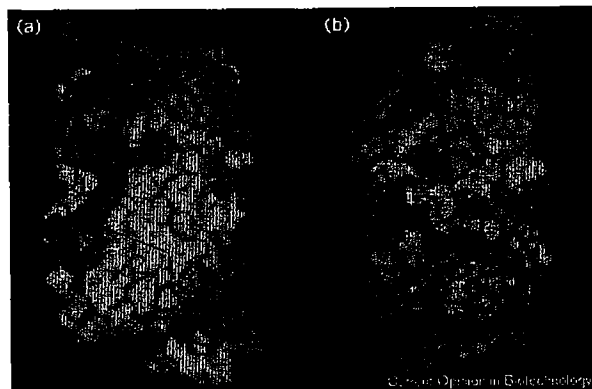
For most steroid receptors, as well as for a number of related nuclear receptors, crystal structures of LBDs have been determined during the past few years; however, not all of them have been published. In addition to the experimentally determined structures, a number of models have been published based on related structures and on a number of additional supporting biological and binding data [25,26*]. An overview of the ER LBD structures and on some relevant models is given in Table 1. The overall structures of the hERα and hERβ LBD-raloxifene complexes are very similar, the root-mean-square deviation between equivalent Cα atoms (excluding helix H12) is 0.97 Å [7].

All known NR LBD structures consist of 11–12 α-helices and one small β-sheet. The secondary structure elements

are arranged in a so-called α-helical sandwich, a protein fold that has up to now only been observed for NR LBDs. This fold is shown as a cartoon in Figure 3 together with the numbering of some of the helices. Helix H2, which has been observed, for example, in the retinoic X receptor (RXR) LBD structure [8] is absent in the hERα LBD. The mainly hydrophobic binding niche, which is described below in more detail, is located in the lower part of the protein with respect to the orientation of the protein shown in Figure 3.

A detailed comparison with the structures of other NR LBDs shows that the upper part of the protein (with respect to the orientation shown in Figure 3) is structurally much better conserved than the lower part harbouring the ligand-binding pocket (LBP) (for details see [3*]; Egner U *et al.*, unpublished data). This can be rationalized as the natural ligands differ considerably in size and shape; the structural variability can thus be attributed to the affinity towards diverse ligands.

Figure 2



Connolly surface of the hER α LBD in complex with (a) estradiol compared to the (b) progesterone receptor LBD. The orientation of the proteins is the same as in Figure 3. Coloring is based on residue conservation: yellow, highly conserved residues (> 80% identity); blue, homologous residues (> 80% similarity); green, no sequence conservation. The red arrow indicates the position of helix H12, the tip of the arrow being the carboxy-terminus of the helix.

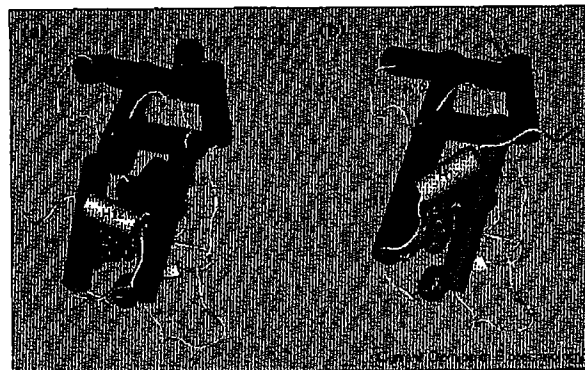
The ligand-binding pocket

ERs bind the endogenous ligand estradiol with a very high affinity of 0.05 nM for hER α and 0.09 nM for hER β , respectively [27]. The protein–ligand interactions have been analyzed and described in detail [28–31,32]. The otherwise hydrophobic LBP, which is not accessible from the protein surface in the agonist-bound state (see below), harbours two polar regions located at opposite ends of the cavity. At the deep end of the pocket, Glu353 and Arg394 (hER α numbering) serve as anchoring points for the 3-hydroxy group of estradiol (E2). In addition, a conserved water molecule stabilizes this interaction. This hydrogen bond network explains the predominant contribution of the 3-hydroxy group to the binding affinity of the steroid [25]. Similar hydrogen bonds are observed in all available hER α and hER β LBD structures.

The 17-hydroxy group of E2 interacts with the second polar region within the LBP, that is, with His524 (hER α numbering). Different sidechain conformations for this residue are observed in the various complex structures known to date. Except for these two polar regions, the LBP is lined by hydrophobic residues. The fact that some are flexible (e.g. methionine and isoleucine) accounts for the high affinity of a variety of steroidal and non-steroidal ligands, including phytoestrogens such as genistein [7,27].

As the estrogen receptors are of such high medical relevance, thousands of ligands have been synthesized within research institutions and in the pharmaceutical industry. With respect to their functions, the ligands can be classified as agonists (e.g. E2 [32*]) partial agonists (e.g. raloxifene [28]) and as pure antagonists [30,32*]. Up to now, structures are only available for the first two groups, so that the structural basis

Figure 3



Cartoon [50] of the hER α LBD in complex with (a) the endogenous ligand estradiol and (b) the partial agonist raloxifene. The helices are represented as barrels. For some of them, the numbering is given.

for the differences between partial agonists and pure antagonists is thus not yet understood.

Conformational changes

For the hER α LBD, seven crystal structures are available. The most striking difference between these structures is the orientation of helix H12. A comparison with related structures ([4,6**]; Ruff M *et al.*, personal communication) indicates that the position of this helix depends on the presence of a ligand. In the only available *apo* form of a NR LBD, the RXR LBD, this helix points away from the protein core [8]. In most of the available agonist-bound LBDs, however, the helix is folded back and serves as a lid closing the entrance to the LBP. At the same time, AF-2 is brought into a position where co-activators can bind resulting in transcription initiation. In the case of the hER α LBD, helix H12 is anchored in this 'agonist conformation' by hydrophobic interactions as well as by hydrogen bonds.

The more space-consuming ER partial agonists with their long-chain substituents cannot be accommodated in the LBP in the 'agonist conformation' of the protein. It was therefore expected that upon partial agonist binding, helix H12 would be present in another orientation. The two different orientations of helix H12 induced by the agonist E2 and the partial agonist raloxifene are depicted in Figure 3, showing that this is the case. Recently, the crystal structure has been determined for the ternary complex of the hER α LBD with the agonist diethylstilbestrol (DES) and a peptide of the co-activator GRIP-1 [6**]. The position of the α -helical peptide almost coincides with helix H12 in the antagonist conformation of the protein: upon superposition, the distance between equivalent C α positions varies between 0.6–2.2 Å. This observation supports the experimental finding that antagonists block co-activator binding.

In contrast to expectation, two structures of the hER α LBD in complex with E2 are presently known where helix

Figure 4

Sequence alignment of hER α , mouse ER α and hER β LBDs. Positions of mutated residues are marked by an (x) below (hER α) or above (mouse ER α) the sequences. Residues belonging to helices in the hER α LBD-estradiol complex are indicated by a gray background; residues in contact with ligands are printed bold face; the numbers of the helices are given.

ER α mouse	312	LSLTADQMV	LLDAEPPMIY	SEY	DPSRPF	SEASMMGLLT	NLAD	355
ER β human	208	DALSPEQLVL	TLLEAPPHV	LIS	RPSAPP	TEASMMSLT	KLAD	250
ER α human	308	LSLTADQMV	LLDAEPPMIY	SEY	DPSRPF	SEASMMGLLT	NLAD	351
Mutations								
Helices			H1			H3		
ER α mouse	356	RELIVHMINWA	KRVPGFGDLN	LHDQVHLLC	AWLEILMIGL	VWRSMEHPG		403
ER β human	251	KELVHMISWA	KKIPGFVELS	LFDQVRLLES	CHMEVIMMGL	MWRSIDHPG		299
ER α human	352	RELIVHMINWA	KRVPGFGDLT	LHDQVHLLC	AWLEILMIGL	VWRSMEHPG		400
Mutations		x	x x x	x x xx	x x x		x x	
Helices				H4		H5		
ER α mouse	404	KLIFAPNLLL	DRNQGKCV	EG	MVEIFDMLLA	TSSRFRMNL	QGEFVCLKS	453
ER β human	300	KLIFAPDLVL	DRDEGKCV	EG	ILEIFDMLLA	TTSRFRMLK	QKEYLCVKA	349
ER α human	401	KLIFAPNLLL	DRNQGKCV	EG	MVEIFDMLLA	TSSRFRMNL	QGEFVCLKS	450
Mutations			x xxx	x x x	x		x	
Helices				H7			H8	
ER α mouse	454	IILLNSGVYT	FLSSTLKSLE	EKDHIHRVLD	KITDTLIHLM	AKAGLTQQQ		503
ER β human	350	MILLNSMYP	LVT	ATQDAD	SSRKLHLLN	AVIDALVWVI	AKSGISSQQ	398
ER α human	451	IILLNSGVYT	FLSSTLKSLE	EKDHIHRVLD	KITDTLIHLM	AKAGLTQQQ		500
Helices				H9			H10	
Mutations		x x x x x		x x x x	x	xxxx	xxxx	
ER α mouse	504	HRRLAQLLLI	LSHIRHMSNK	GMEHLYNMKC	KNVVPYDILL	LEMLDAH	RLH	553
ER β human	399	SMRLANLLML	LSHVRHASNK	GMEHLINMKC	KNVVPYDILL	LEMLDAH	VLR	448
ER α human	501	HRRLAQLLLI	LSHIRHMSNK	GMEHLYNMKC	KNVVPYDILL	LEMLDAH	RLH	550
Mutations		x	xxxxxx	xxxxxxxx	xxxxxxxx	xxxxxx	x x x	
Helices		H10		H11		H12		

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H12 is not in the agonist conformation. In the artificial tetramer crystallized by Tannenbaum *et al.* [5], this helix interacts with a neighboring LBD molecule, and in the recently crystallized triple mutant (Ruff M *et al.*, personal communication), the helix is in the 'partial agonist conformation'. The meaning of this structural variability is not yet fully understood but might be caused by the absence of the carboxy-terminal F-domain (adjacent to helix H12) of the hER in all crystal structures. There is no experimental evidence that the structural variability observed in the structures is caused by the absence of the F-domain. It can not, however, be excluded that an additional domain has a stability effect on three dimensional structure.

Differences between receptor subtypes

The tissue distribution of hER α and hER β was investigated shortly after the discovery of the second isoform [16]. hER β is the predominant subtype in bone, blood vessels and in the brain, whereas its content in uterus and liver is only low. Because of the different distribution in the various target organs, the medical interest in subtype-specific ligands is high.

Both human receptors share ~55% identical amino acid residues in their LBDs. The analysis of the LBPs shows that most residues in direct contact with E2 in the hER α LBD are conserved in hER β . The same is true for residues in the second shell around the ligand. There are a number of differences, however, in the vicinity of the steroidal A-ring

close to position 4 of estradiol. In this area, six homologous replacements are encountered (e.g. Ile→Leu, Met or Val). Another two homologous exchanges are present in the environment of the steroidal D-ring. In spite of the high degree of conservation between the LBPs, an ER α subtype-specific ligand exhibiting a more than 100-fold difference in binding affinity has recently been published [31].

Dimerization and binding of co-activators/co-repressors

Receptor dimerization is a prerequisite for transcription initiation in ER-regulated processes. As LBDs contribute to the dimerization interface, it was expected that the hER α -E2 complex would crystallize as a dimer, as is the case. There is evidence that the interactions observed in the crystals reflect the natural dimerization contacts: firstly, all published point mutants influencing ER dimerization are in, or at least close to, a region involved in crystal contact; and secondly, dimers have been observed in crystal structures of related LBDs ([4,6**]; Ruff M *et al.*, personal communication). The topology of the dimer interfaces is comparable in all these structures, especially concerning the key role of helix H10 in the intermolecular contacts. In the case of the hER α -E2 complex, the monomer-monomer interactions are very strong: 27 amino acid residues contribute to the large contact surface and out of these 14 are involved in hydrogen bonds.

In recent years, several novel classes of transcriptional mediators (co-activators and co-repressors) that interact

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Table 1

Published crystal structures of hER α and hER β LBDs.

LBD	Ligand/co-activator peptide			Resolution (Å)	Relative position of helix H12
	Endogenous ligand/agonist	Partial agonist/antagonist	Co-activator peptide		
hERα LBD					
LBD carboxy-methylated [6**]	DES		GRIP-1: 686-698	2.0	
Wild-type LBD (a)	Estradiol			3.2	
LBD carboxy-methylated [4]	Estradiol			3.1	
Mutant LBD: C381S/C417S/C530S (a)	Estradiol			2.2	
LBD carboxy-methylated [6**]		Tamoxifen		1.9	
LBD carboxy-methylated [4]		Raloxifene		2.6	
Tetramer due to intermolecular disulfide bond [5]	Estradiol			2.8	
Model [25]	Estradiol and others			Not applicable	
Model [26*]	Estradiol and others			Not applicable	
hERβ LBD [7]					
LBD carboxy-methylated		Genistein		1.8	
		Raloxifene		2.25	

(a) M Ruff, M Gangloff, M Eiler, S Duclaud, JM Wurtz, D Moras, unpublished data. DES, diethylstilbestrol.

with LBDs have been identified and characterized [33-35,36*,37-39]. It should be mentioned here that some of these mediators, such as NSD1 and GRIP-1, are ligand dependent [33,36*], whereas for others, such as SRC-1, a hormone-independent mechanism is discussed [39]. The recently elucidated structure of hER α LBD in complex with a GRIP-1 peptide has already been mentioned above [6**]; however, a comparable complex structure with a hormone-independent mediator is not yet available.

Mutations

In contrast to, for example, the androgen receptor, only one germline mutation is known for the hER, which led to osteoporosis in a young man [40]. A number of splice variants have been identified on the mRNA level and their biological role remains to be elucidated. Furthermore, some point mutations have been identified in patients with primary breast cancer [41].

For a better understanding of the functional role of different residues, a number of engineered mutants of the hER α LBD have been investigated ([26*] and references therein, [41-48]), but only four mutants for hER β LBD have yet

been published. Positions of mutated residues are indicated in the sequence alignment shown in Figure 4. For a number of these mutants, the influence on ligand binding has been analyzed in detail (e.g. [43], and references in [26*]). For the vast majority of them the influence on the binding activity could be understood on the basis of the hER α LBD structure. In the more recent literature, the effect of mutants on the transcription potency and on the interaction with co-activators/co-repressors is also described (e.g. [44,46]).

Conclusions

During the past few years, the progress in our understanding of the hER α LBD actions has been tremendous. Several scientific areas have contributed to this achievement. Firstly, after more than one decade of intensive work, crystal structures of hER α and hER β LBD complexes, as well as of related NR LBDs, are now available. Secondly, novel classes of co-activators and co-repressors have been identified and characterized, which have been mentioned only briefly in this review. Thirdly, new steroidal and non-steroidal ligands to the hERs have been synthesized. A special emphasis has been on the search for pure antagonists, which are considered to have an

improved clinical profile as compared to the commonly used partial agonists, such as tamoxifen. Finally, the effect of engineered mutants has been studied in detail and supported our understanding of the role of single residues within the protein subunit.

In spite of the progress outlined above, many open questions and uncertainties remain concerning the ER LBD actions. To give one example, no crystal structure is available for a complex with a pure antagonist, that is, the structural basis for the functional differences between partial agonists and pure antagonists is unknown. Furthermore, the role of helix H12 and its different orientations has in part been deduced from crystal structures in which the domain adjacent to helix H12, the F-domain, is missing. The conformational variability of this helix might thus be an artefact depending upon the choice of the protein fragment used for the crystallization trials.

If the number of new ER LBD structures further increases at its current rate and if the information about the co-activators and co-repressors continues to contribute equally fast to our understanding of ER actions, we will soon be able to form a realistic picture on the early stages of transcriptional activation.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Katzenellenbogen BS: **Estrogen receptors: bioactivities and interaction with cell signalling pathways.** *Biol Reprod* 1996, 54:287-293.
2. Ciocca DR, Roig LM: **Estrogen receptors in human nontarget tissues: biological and clinical implications.** *Endocrinol Rev* 1995, 16:35-62.
3. Moras D, Gronemeyer H: **The nuclear receptor ligand-binding domain: structure and function.** *Curr Opin Cell Biol* 1998, 10:384-391. The authors give an overview of different nuclear receptor ligand-binding domains structures in a condensed form. The most relevant information on co-activators/co-repressors is also presented.
4. Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engström O, Ohman L, Green GL, Gustafsson JA, Carlquist M: **Molecular basis of agonism and antagonism in the oestrogen receptor.** *Nature* 1997, 389:753-758.
5. Tannenbaum DM, Wang Y, Williams SP, Sigler PB: **Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains.** *Proc Natl Acad Sci USA* 1998, 95:5998-6003.
6. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL: **The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen.** *Cell* 1998, 95:927-937. The first example of a crystal structure of a ternary complex of the hERα ligand-binding domain (LBD) in complex with an agonist (diethylstilbestrol) and a co-activator fragment. The crystal structure of the hER LBD in complex with a partial agonist, tamoxifen, is also presented. The structural basis for co-activator recognition is also discussed with respect to the interaction of tamoxifen with the protein.
7. Pike ACW, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engström O, Ljunggren J, Gustafsson JA, Carlquist M: **Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full agonist.** *EMBO J* 1999, 18:4608-4618.
8. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D: **Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-α.** *Nature* 1995, 375:377-382.
9. Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D: **Crystal structure of the RAR-γ ligand-binding domain bound to all-trans retinoic acid.** *Nature* 1995, 378:681-689.
10. Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ: **A structural role for hormone in the thyroid hormone receptor.** *Nature* 1996, 378:690-697.
11. Ribeiro RCJ, Apriletti JW, Wagner RL, Feng W, Kushner PJ, Nilsson S, Scanlan TS, West BL, Fletterick RJ, Baxter JD: **X-ray crystallographic and functional studies of thyroid hormone receptor.** *J Steroid Biochem Molec Biol* 1998, 65:133-141.
12. Williams SP, Sigler PB: **Atomic structure of progesterone complexed with its receptor.** *Nature* 1998, 393:392-396.
13. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV: **Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ.** *Nature* 1998, 395:137-143.
14. Uppenberg J, Svensson C, Jaki M, Bertilsson G, Jendberg L, Berkenstam A: **Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ.** *J Biol Chem* 1998, 273:31108-31112.
15. Klaholz BP, Renaud JP, Mitschler A, Zusi C, Chambon P, Gronemeyer H, Moras D: **Conformational adaption of agonists to the human nuclear receptor RARγ.** *Nat Struct Biol* 1998, 5:199-202.
16. Kuiper GGJM, Enmark E, Peltö-Huikko M, Nilsson S, Gustafsson JA: **Cloning of a novel estrogen receptor expressed in rat prostate and ovary.** *Proc Natl Acad Sci USA* 1996, 93:5925-5930.
17. Giguere V, Yang N, Segui P, Evans RM: **Identification of a new class of steroid hormone receptors.** *Nature* 1988, 331:91-94.
18. Saunders P: **Oestrogen receptor beta (ERβ).** *Rev Reprod* 1998, 3:154-171.
19. Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Home EL, Su JL, Kiewer SA, Lehmann JM, Willson TM: **Cloning and characterization of human estrogen receptor β isoforms.** *Biochem Biophys Res Commun* 1998, 247:75-78.
20. Vladusik EA, Homby AE, Guerra Vladusik FK, Lupu R: **Expression of estrogen receptor beta messenger RNA variant in breast cancer.** *Cancer Res* 1998, 58:210-214.
21. Petersen DN, Tkalecic GT, Koza-Taylor PH, Turi TG, Brown TA: **Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues.** *Endocrinology* 1998, 139:1082-1092.
22. Maruyama K, Endoh H, Sasaki-Iwaoaka H, Kanou H, Shimaya E, Hashimoto S, Kato S, Kawashima H: **A novel isoform of rat estrogen receptor beta with 18 amino acid insertion in the ligand binding domain as a putative dominant negative regulator of estrogen action.** *Biochem Biophys Res Commun* 1998, 248:142-147.
23. Murphy LC, Dotzlaw H, Leygue E, Douglas D, Coutts A, Watson PH: **Estrogen receptor variants and mutations.** *J Steroid Biochem Mol Biol* 1997, 62:363-372.
24. Bairoch A, Apweiler R: **The SWISS-PROT protein sequence data bank and its supplement TrEMBL in 1999.** *Nucleic Acid Res* 1999, 27:49-54.
25. Anstead GM, Carlson KE, Katzenellenbogen JA: **The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site.** *Steroids* 1997, 62:268-303.
26. Wurtz JM, Egner U, Heinrich N, Moras D, Mueller-Fahmow A: **Three dimensional models of estrogen receptor ligand binding domain complexes, based on related crystal structures and mutational and structure-activity relationship data.** *J Med Chem* 1998, 11:1803-1814. Thorough comparative modelling study. The hERα ligand-binding domain (LBD) model is evaluated based on the analysis of ligands and their relative binding affinities. The article provides a list of mutants within the LBD of human and mouse estrogen receptor evaluating the binding affinities with respect to the wild-type receptor.

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27. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology* 1998, 139:4252-4263.
28. Gress TA, Dodge JA: Selective estrogen receptor modulators (SERMs). *Curr Pharmaceut Des* 1998, 4:71-92.
29. Levenson AS, Jordan VC: The key to antiestrogenic mechanism of raloxifene is amino acid 351 (aspartate) in the estrogen receptor. *Cancer Res* 1998, 58:1872-1875.
30. Macgregor JJ, Jordan VC: Basic guide to the mechanism of antiestrogen action. *Pharmacol Rev* 1998, 50:151-196.
31. Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS: Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor- α or estrogen receptor- β . *Endocrinology* 1999, 140:800-804.
32. Gao H, Katzenellenbogen JA, Garg R, Hansch C: Comparative QSAR analysis of estrogen receptor ligands. *Chem Rev* 1999, 99:723-744.
This study provides detailed information on comparative structure-activity relationships of steroidal and non-steroidal estrogen receptor ligands. Factors influencing the relative binding affinity, for example, the steric and polar character of substituents are highlighted. The quality of the derived relationships is critically analysed.
33. Huang N, von Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R: Two distinct nuclear receptor interaction domains in NRD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *EMBO J* 1998, 17:3398-3412.
34. vom Baur E, Harbers M, Um SJ, Benecke A, Chambon P, Losson R: The yeast Ada complex mediates the ligand-dependent activation function AF-2 of retinoid X and estrogen receptors. *Genes Dev* 1998, 12:1278-1289.
35. Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson JA, Treuter E: The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activation of estrogen receptors ER α and ER β . *J Biol Chem* 1999, 274:345-353.
36. Norris JD, Fan D, Stallcup MR, McDonnell DP: Enhancement of estrogen receptor transcriptional activity by the coactivator GRIP-1 highlights the role of activation function 2 in determining estrogen receptor pharmacology. *J Biol Chem* 1998, 273:6679-6688.
The authors analyze the interaction of the co-activator GRIP1 with the estrogen receptor. They suggest that GRIP1 may not only interact with the trans-activation function 2 (AF-2) of the ligand-binding domain (LBD) but also contacts the receptor in yet unidentified areas of the LBD. The results are supported by mutagenesis experiments of selected amino acid residues in helix H12 of the LBD.
37. Peters GA, Khan SA: Estrogen receptor domains E and F: role in dimerisation and interaction with coactivator RIP-140. *Mol Endocrinol* 1998, 13:286-296.
38. Parker MG: Transcriptional activation by oestrogen receptors. *Biochem Soc Symp* 1998, 63:45-50.
39. Smith CL, Nawaz Z, O'Malley BW: Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Mol Endocrinol* 1997, 11:657-666.
40. Smith EP, Boyd J, Frank G, Takahashi H, Cohen R, Specker B, Williams T, Lubahn D, Korach K: Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. *N Engl J Med* 1994, 331:1056-1061.
41. Umekita Y, Sagara Y, Yoshida H: Estrogen receptor mutations and changes in estrogen receptor and progesterone receptor protein expression in metastatic or recurrent breast cancer. *Jpn J Cancer Res* 1998, 89:27-32.
42. Nichols M, Rientjes JMJ, Stewart AF: Different positioning of the ligand-binding domain helix 12 and the F domain of the estrogen receptor accounts for functional differences between agonists and antagonists. *EMBO J* 1998, 17:765-773.
43. Ekena K, Katzenellenbogen JA, Katzenellenbogen BS: Determinants of ligand binding specificity of estrogen receptor- α : estrogen versus androgen discrimination. *J Biol Chem* 1998, 273:693-699.
44. Tremblay GB, Tremblay A, Labrie F, Giguère V: Ligand-independent activation of the estrogen receptors α and β by mutations of a conserved tyrosine can be abolished by antiestrogens. *Cancer Res* 1998, 58:877-881.
45. Tremblay GB, Tremblay A, Labrie F, Giguère V: Dominant activity of activation function 1 (AF-1) and differential stoichiometric requirements for AF-1 and -2 in the estrogen receptor α - β heterodimeric complex. *Mol Cell Biol* 1999, 19:1919-1927.
46. Feng W, Ribeiro RCJ, Wagner RL, Nguyen H, Apriletti JW, Fletterick RJ, Baxter JD, Kushner PJ, West BL: Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. *Science* 1998, 280:1747-1749.
47. Taylor JA, Lewis KJ, Lubahn DB: Estrogen receptor mutations. *Mol Cell Endocrinol* 1998, 145:61-66.
48. Miller N, Whelan J: Random mutagenesis of human estrogen receptor ligand binding domain identifies mutations that decrease sensitivity to estradiol and increase sensitivity to a diphenol indene-ol compound: basis for a regulatable expression system. *J Steroid Biochem Mol Biol* 1998, 64:129-135.
49. Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res* 1994, 22:4673-4680.
50. Insight98.0. San Diego, USA: MSI Inc.; 1998.